

In the Claims

Please amend the second occurrence of independent claim 16 and claims 17-62. The amended claims are provided below in clean form. Per 37 C.F.R. §1.121, amended claims are also shown in Appendix A with notations to indicate changes made (for convenience, all pending claims, including those added hereby, are provided in Appendix A).

~~17. (Amended) A solid support comprising at least one oligonucleotide selected from the group consisting of an oligonucleotide comprising a phosphoroselenoate group, an oligonucleotide comprising phosphoroselenoate group, an oligonucleotide comprising a phosphorotelluroate group, and an oligonucleotide comprising a 5' leaving group.~~

18. (Amended) The solid support of claim 17 comprising an oligonucleotide comprising a 5' leaving group and at least one oligonucleotide selected from the group consisting of an oligonucleotide comprising a phosphoroselenoate group, an oligonucleotide comprising phosphoroselenoate group, an oligonucleotide comprising a phosphorotelluroate group.

19. (Amended) A method for making an oligonucleotide comprising:
binding at least one upstream oligonucleotide and at least one downstream oligonucleotide to a polynucleotide template;
the upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group; and
the downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' phosphoroselenoate or a 3' phosphorotelluroate, wherein the downstream oligonucleotide binds such that its 3' end is substantially adjacent to the 5' end of the upstream oligonucleotide;
to yield an autoligated oligonucleotide product comprising the upstream oligonucleotide ligated to the downstream oligonucleotide.

20. **(Amended)** The method of claim 19 where one oligonucleotide comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group, and wherein the presence or absence of the autoligated oligonucleotide product is accompanied by a detectable change in fluorescence emission of the ligated product compared to the fluorescence energy emissions of the unligated oligonucleotides.

21. **(Amended)** A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' phosphoroselenoate or a 3' phosphorotelluroate, such that, when both probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' phosphoroselenoate or a 3' phosphorotelluroate in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and

detecting the presence of the autoligated oligonucleotide product.

22. **(Amended)** The method of claim 21 wherein at least one of the mutant polymorphism oligonucleotide probe and the universal oligonucleotide probe comprises a detectable label.

23. (Amended) The method of claim 22 wherein the detectable label is a radiolabel.
24. (Amended) The method of claim 21 wherein the genetic polymorphism is selected from the group consisting of a single base mutation, a plurality of single base mutations, a deletion, an insertion, and a genetic rearrangement.
25. (Amended) The method of claim 21 wherein the nucleotide position of the genetic polymorphism is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.
26. (Amended) The method of claim 21 wherein the mutant polymorphism probe is about 3 to about 12 nucleotides in length.
27. (Amended) The method of claim 26 wherein the mutant polymorphism probe is about 3 to about 6 nucleotides in length.
28. (Amended) The method of claim 21 wherein the target polynucleotide is DNA or RNA.
29. (Amended) The method of claim 21 wherein the target polynucleotide is double-stranded or single-stranded.
30. (Amended) The method of claim 21 wherein one oligonucleotide probe comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group, and wherein the presence or absence of the autoligated oligonucleotide product is accompanied by a detectable change in fluorescence emission of the ligated product compared to the fluorescence emissions of the unligated oligonucleotides.

31. (Amended) A method for determining whether a target polynucleotide contains a genetic polymorphism comprising:

providing a mutant polymorphism oligonucleotide probe comprising a first fluorescence energy acceptor group, wherein the mutant polymorphism oligonucleotide probe is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a wild-type polymorphism oligonucleotide probe comprising a second fluorescence energy acceptor group, wherein the wild-type polymorphism oligonucleotide probe is complementary to a region on the analogous wild-type polynucleotide that is analogous to the region comprising the genetic polymorphism;

providing a universal oligonucleotide probe comprising a fluorescence energy donor group, wherein the universal probe is capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein either (i) the universal oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and both polymorphism oligonucleotide probes constitute downstream oligonucleotides comprising, as their 3' ends, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate; or (ii) both polymorphism oligonucleotide probes constitute upstream oligonucleotides comprising, as their 5' ends, a nucleoside comprising a 5' leaving group and the universal oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate;

such that, when a universal probe and a polymorphism probe are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' functional group in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe, the mutant polymorphism oligonucleotide probe and the wild-type polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe either the mutant polymorphism probe or the wild-type polymorphism oligonucleotide probe; causing the autoligated oligonucleotide product to fluoresce; and analyzing the fluorescence emission from the autoligated oligonucleotide product to determine whether the autoligated oligonucleotide product comprises the mutant polymorphism probe or the wild-type polymorphism oligonucleotide probe, wherein the presence of the mutant polymorphism probe in the autoligated oligonucleotide product indicates the presence of a genetic polymorphism in the target polynucleotide.

138 32. **(Amended)** The method of claim 31 wherein the genetic polymorphism is selected from the group consisting of a single base mutation, a plurality of single base mutations, a deletion, an insertion, and a genetic rearrangement.

33. **(Amended)** The method of claim 31 wherein the nucleotide position of the genetic polymorphism is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.

34. **(Amended)** The method of claim 31 wherein the mutant polymorphism probe is about 3 to about 12 nucleotides in length.

35. **(Amended)** The method of claim 34 wherein the mutant polymorphism probe is about 3 to about 6 nucleotides in length.

36. **(Amended)** The method of claim 31 wherein the target polynucleotide is DNA or RNA.

37. **(Amended)** The method of claim 31 wherein the target polynucleotide is single-stranded or double-stranded.

38. **(Amended)** A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate, such that, when both probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' functional group in close proximity to one another;

and wherein one oligonucleotide probe comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and

detecting the presence or absence of the autoligated oligonucleotide product, wherein the presence or absence of the autoligated oligonucleotide product is accompanied by a detectable change in fluorescence emission of the ligated product compared to the fluorescence emissions of the unligated oligonucleotides.

39. (Amended) The method of claim 38 wherein the nucleotide position of the genetic polymorphism is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.

40. (Amended) The method of claim 38 wherein the mutant polymorphism probe is about 3 to about 12 nucleotides in length.

41. (Amended) The method of claim 40 wherein the mutant polymorphism probe is about 3 to about 6 nucleotides in length.

42. (Amended) The method of claim 38 wherein the target polynucleotide is DNA or RNA.

43. (Amended) The method of claim 38 wherein the target polynucleotide is single-stranded or double-stranded.

44. (Amended) A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate, such that, when both probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially but not directly adjacent to an end of the mutant polymorphism oligonucleotide

probe so as to position the 5' leaving group and the 3' functional group in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and
detecting the presence of the autoligated oligonucleotide product.

45. **(Amended)** The method of claim 44 wherein at least one of the mutant polymorphism oligonucleotide probe and the universal oligonucleotide probe comprises a detectable label.

46. **(Amended)** The method of claim 45 wherein the detectable label is a radiolabel.

47. **(Amended)** The method of claim 44 wherein the genetic polymorphism is selected from the group consisting of a single base mutation, a plurality of single base mutations, a deletion, an insertion, and a genetic rearrangement.

48. **(Amended)** The method of claim 44 wherein the nucleotide position of the genetic polymorphism is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.

49. **(Amended)** The method of claim 44 where one oligonucleotide comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group, and wherein the presence or absence of the autoligated oligonucleotide product is accompanied by a detectable change in fluorescence emission of the ligated product compared to the fluorescence energy emissions of the unligated oligonucleotides.

50. **(Amended)** A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe of less than 7 nucleotides in length that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate, such that, when both probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' functional in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and

detecting the presence of the autoligated oligonucleotide product.

51. **(Amended)** The method of claim 50 wherein at least one of the mutant polymorphism oligonucleotide probe and the universal oligonucleotide probe comprises a detectable label.

52. **(Amended)** The method of claim 51 wherein the detectable label is a radiolabel.

53. **(Amended)** The method of claim 50 wherein the genetic polymorphism is selected from the group consisting of a single base mutation, a plurality of single base mutations, a deletion, an insertion, and a genetic rearrangement.

54. **(Amended)** The method of claim 50 wherein the nucleotide position of the genetic polymorphism is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.

55. **(Amended)** The method of claim 50 where one oligonucleotide comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group, and wherein the presence or absence of the autoligated oligonucleotide product is accompanied by a detectable change in fluorescence emission of the ligated product compared to the fluorescence energy emissions of the unligated oligonucleotides.

As 56. **(Amended)** A method for detecting a genetic polymorphism in a target RNA comprising:
providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target RNA that comprises the genetic polymorphism;
providing a universal oligonucleotide probe capable of binding to the target RNA at a region that is conserved in the analogous wild-type RNA;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate, such that, when both probes are bound to the target RNA, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' functional group in close proximity to one another;

contacting the target RNA with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and

detecting the presence of the autoligated oligonucleotide product.

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*Title: COMPOSITIONS AND METHODS FOR NONENZYMATIC LIGATION OF OLIGONUCLEOTIDES AND
DETECTION OF GENETIC POLYMORPHISMS*

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~~57. (Amended) The method of claim 56 wherein at least one of the mutant polymorphism oligonucleotide probe and the universal oligonucleotide probe comprises a detectable label.~~

58. (Amended) The method of claim 57 wherein the detectable label is a radiolabel.

59. (Amended) The method of claim 56 wherein the genetic polymorphism is selected from the group consisting of a single base mutation, a plurality of single base mutations, a deletion, an insertion, and a genetic rearrangement.

60. (Amended) The method of claim 56 wherein the nucleotide position is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.

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61. (Amended) The method of claim 56 where one oligonucleotide comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group, and wherein the presence or absence of the autoligated oligonucleotide product is accompanied by a detectable change in fluorescence emission of the ligated product compared to the fluorescence energy emissions of the unligated oligonucleotides.

62. (Amended) A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' phosphoroselenoate or a 3' phosphorotelluroate, such that, when both

probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' phosphoroselenoate or a 3' phosphorotelluroate in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and

detecting the presence of the autoligated oligonucleotide product;
wherein the autoligation is reversible by contacting the autoligated oligonucleotide product with silver or mercuric ions.

63. **(Amended)** A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a 5'-iodopyrene and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a pyrene nucleoside selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate, such that, when both probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5'-iodopyrene and the 3' pyrene nucleoside in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product

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comprising the universal oligonucleotide probe, the mutant polymorphism probe, and a pyrene
excimer; and

detecting the presence of the autoligated oligonucleotide product using excimers as
labels.
